United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Effect of atmospheric smog on cardiopulmonary responses in rats
LAPR Number:	19-04-001
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6/RTP/USEPA/US
Date Originated:	03/29/2016
LAPR Expiration Date:	03/29/2019
Agenda Date:	04/06/2016
Date Approved:	04/19/2016
Date Closed:	

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	04/19/2016	DMR	
	Exemption 6 Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	04/19/2016	DMR approved	

Administrative Information

1. Project Title (no abbreviations, include species):
Effect of atmospheric smog on cardiopulmonary responses in rats

Is this a continuing study with a previously approved LAPR?
No

2. Programatic Information

a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.
Air Climate and Energy (ACE), PEP2.1 - Relationships between the potency of different atmospheric smog mixtures on health.

b. What is the Quality Assurance Project Plan (QAPP) covering this project?
IRP-NHEERL-RTP/EPHD/CIB[redacted]/2014-001-r1

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6	EPHD	MD
	Lotus Notes Address	*Branch*	
	[redacted]/RTP/USEPA/US	CIB	

4. Alternate Contact:

Alternate Contact	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6	EPHD	MD
	Lotus Notes Address	*Branch*	
	[redacted]RTP/USEPA/US	CIB	

SECTION A - Description of Project

1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

Ambient air pollution is a mixture of many different components including particulate matter (PM), gaseous irritants, volatile organic compounds as well as other substances like biologicals. Although it has become clear

that exposure to air pollution causes deleterious cardiopulmonary effects, the relative potency of a given air pollution mixture (when compared to another) is not entirely known. Thus, assessments of air pollution effects have grown from studying exposures to single agents such as ozone and PM to investigating more complex mixtures from distinct sources such as diesel or gasoline exhaust. Indeed, the National Academy of Science (NAS) and National Research Council (NRC) have stated that "now is an opportune time to begin orienting EPA's air research program toward a broader scope that specifically considers all components of the atmosphere-PM and the other criteria pollutants, hazardous pollutants, and the other non-classified components of the atmosphere."

To quantify the health effects of multi-pollutant atmospheres in a controlled setting, we will utilize a controlled static and dynamic chamber (mobile reaction chamber: [MRC]) that can accommodate the mixing of primary emissions from sources including diesel and gasoline (raw fuel or combusted emissions from generators), biogenic hydrocarbons (e.g. alpha-pinene) and inclusion of oxidant substrates (e.g. NO), with subsequent photochemical aging to produce stable atmospheres of urban smog. Several distinct scenarios will be produced to achieve worsening degrees of air quality. Specifically we will generate atmospheres that have an air quality index (AQI) dominated by high PM2.5 and/or high oxidants (ozone - O3 and nitrogen dioxide - NO2) as well as other combinations of reactants that cause varying concentrations of PM or ozone.

As far as health effects are concerned, the objective of this project is to determine the effects of one or two days of smog exposure on cardiovascular dysfunction in normal (Wistar-Kyoto - WKY) and spontaneously hypertensive (SH) rats. This project will assess cardiovascular responses using a Millar intravascular probe to measure ventricular function which will include pharmacological challenge with a sympathomimetic drug (i.e. dobutamine) and the aconitine (arrhythmia) challenge testing. We hypothesize different smog atmospheres will exacerbate adverse cardiopulmonary responses in rats to a varying degree and that SH rats will have a worse response than WKY. Mechanisms related to the transient receptor potential A1 (TRPA1) channel will also be investigated given we have previously shown that it mediates air pollution-induced cardiovascular dysfunction.

The knowledge gained from these studies will not only contribute to our understanding of air pollution health effects but also the relative importance of particular air pollution constituents and the their impact at various concentrations. The use of rats in these types of toxicological studies is necessary because it will provide valuable physiological data (heart rate, ventilation, cardiac function) which directly parallels human responses and public health. In any case, the studies outlined here will provide valuable information on the deleterious health effects of air pollution exposure, the physiological mechanism that causes the dysfunction and risk assessment and mitigation strategies.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

The adverse effects ascribed to air pollution exposure result from the interplay and summation of responses across multiple organ systems, with one deficient organ system impacting others. Similarly, underlying cardiovascular disease (e.g. hypertension) can worsen the overall response to air pollution; thus, it is necessary that whole animals be used for these studies to examine the systemic physiology and changes in homeostatic controls.

b. Justify the species requested:

1) To fully establish the comparative health effects of different smog atmospheres, rats are necessary and represent a good model from which human responses can be extrapolated. For instance, air pollution-induced cardiovascular deficits in humans and rats, which includes increased blood pressure, arrhythmia and functional deficits, are similar (Peters et al. 2000; **Exemption 6** Langrish et al. 2014).
2) There is a very large toxicological database using the rat as the subject species and this will provide the capability to compare toxicity data within a given species.
3) Numerous studies have examined the impact of complex air pollution mixtures on physiological responses in rats and the increased susceptibility of adverse health effects, however none have compared the variable effects due to different smog atmospheres.

3. How was it determined that this study is not unnecessary duplication?

Searches of the entire air pollution health research database via MedLine and Pub-Med (key words: rat, air pollution, cardiac, pulmonary, susceptibility, TRPA1), as well as the current Integrated Scientific Assessment document for Particulate Matter, Ozone and Nitrogen Oxides have yielded no previous studies similar to those

proposed below (i.e. determining the comparative effects of different smog atmospheres) or any concurrent work that is similar; thus, these studies will not duplicate any previously conducted research.

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

The studies described here will examine the effects of various atmospheric smog mixtures (below), which will be characterized by the concentration of PM and oxidant gases (i.e. O3) or the "parent" compound they are generated from. The latter is important given the overall composition of a given smog atmosphere varies based on its source. All studies will use male Wistar-Kyoto (WKY) and/or spontaneously hypertensive (SH) rats. For each study below, there will be two experimental procedures performed on separate animals in each group (i.e. filtered air or smog-exposed): (A) Intravascular/ventricular (Millar) functional testing using dobutamine challenge; (B) aconitine arrhythmia challenge. All animals will be acquired from Charles River Laboratories at 10-12 weeks of age.

Atmosphere 1: The source will be isoprene/gasoline with high PM and low ozone.

Atmosphere 2: The source will be isoprene/gasoline with low PM and high ozone.

Atmosphere 3: The source will be alpha-pinene only.

Atmosphere 4: The source will be isoprene only.

Atmosphere 5: The source will be m-xylene/toluene only.

Atmosphere 6: The source will be t-2-pentene only.

Atmosphere 7: The source will be 2,2,4-Trimethylpentane only.

Atmosphere 8: The source will be n-pentane only.

NOTE: Animals will not be exposed to the source materials but rather the PM, oxidant gases, and other reactant species that are generated from their reaction in the MRC.

All studies - Animals will not be implanted with radiotelemeters; instead the telemeter will be fastened externally after anesthesia and thereafter the animals will be surgically implanted with an intravenous catheter just before experimentation, which will be terminal. Catheterization is a Category D procedure - please see section B7a for complete description of this surgical procedure. All animals will be monitored every other day visually for obvious distress or changes in gait, breathing, appetite, etc.

The role of TRPA1 will be examined through the use of a pharmacological agent, HC030031 (TRPA1 antagonist), in all of the above studies.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

An outline of the studies, animal numbers, procedures and categories is below. A sample size of 8 animals for the intravascular/ventricular (Millar) functional testing with dobutamine will allow for sufficient power in the statistical analysis of various biological/molecular and physiological (electrocardiogram, blood pressure, heart rate, contractility, and arrhythmia) endpoints.We will have a sample size of 6 per group for the aconitine

arrhythmia sensitivity test because an n of 6 is the number of animals that can be reasonably expected to be completed in one day (surgery and procedure take over an hour for each animal) while maintaining sufficient statistical power. Studies will consist of one 4-hour exposure with the following two separate procedures:
A = Intravascular/ventricular (Millar) functional testing with dobutamine
B = Aconitine arrhythmia challenge test

BASIC STUDY DESIGN: this will be repeated for the 8 different exposure atmosphere conditions
A. Air - n = 8 rats/group x 2 treatments (saline or HC030031) x 2 exposure periods (one or two days) x 2 strains (WKY or SH) = 64
Smog - n = 8 rats/group x 2 treatments (saline or HC030031) x 2 exposure periods (one or two days) x 2 strains (WKY or SH) = 64
B. Air - n = 6 rats/group x 2 treatments (saline or HC030031) x 2 exposure periods (one or two days) x 2 strains (WKY or SH) = 48
Smog - n = 6 rats/group x 2 treatments (saline or HC030031) x 2 exposure periods (one or two days) x 2 strains (WKY or SH) = 48

Total for each exposure atmosphere condition = 224

Atmosphere	Number of animals
Isoprene/gasoline (high PM/low ozone)	224
Isoprene/gasoline (low PM/high ozone)	224
alpha-pinene	224
Isoprene	224
m-xylene/toluene	224
t-2-pentene	224
2,2,4-Trimethylpentane	224
n-pentane	224

TOTAL NUMBER OF ANIMALS = 1792 (All untelemetered - Category D)

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:		
D) Potential pain/distress relieved by appropriate measures:	1792	
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:

☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☒ Non-survival surgery

a. Please provide a scientific justification. Describe how animals will be monitored, how health status will be tracked, and what records will be maintained.

All animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily. The personnel responsible for monitoring include: Exemption 6Exemption 6Exemption 6Exemption 6 Exemption 6 Exemption 6. Animals will be weighed every 3 days and tracked for sudden weight loss (>10%). If signs of distress or other deleterious effects are observed, all animals from the treatment group will be isolated in a clean control atmosphere and observed for recovery trends. They may be reused for the study if recovery is demonstrated; otherwise, they will be euthanized. The attending veterinarian will be consulted when appropriate to determine the appropriate course of action. The personnel listed above will be responsible for monitoring animals during holidays and weekends, and a health report will be maintained for each effected animal.

Use of the intravascular/ventricular Millar probe to measure blood pressure/left ventricular function is the only way to determine cardiac mechanical responses in the whole anesthetized animal. The use of this method allows us to examine the contribution of the autonomic nervous system as well as any circulating mediators as opposed to an ex-vivo system like the excised cardiac reperfusion method. It also allows for the use of a sympathomimetic

agent (dobutamine) that increases the heart rate, contractility and blood pressure and reveals suble air pollution effects which may not be evident otherwise. The above reasoning also applies to the aconitine arrhythmia challenge test which can only be performed in the whole anesthetized animal and has already been shown to reveal latent susceptibility to arrhythmia Exemption 6 Anesthetized animals will be monitored during the procedure and should they show any signs of recovery from anesthesia (e.g. eye and body movement) then the animal will be euthanized by overdose of sodium pentobarbital (200 mg/kg, i.p.) followed by organ resection.

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

Smog Inhalation

To quantify the health effects of multi-pollutant atmospheres in a controlled setting, we will utilize a new controlled static and dynamic chamber (mobile reaction chamber: [MRC]) that can accommodate the mixing of primary emissions from sources including diesel and gasoline, biogenic hydrocarbons and inclusion of oxidant substrates (e.g. NO), with subsequent photochemical aging to produce stable atmospheres of urban smog. The smog mixture will contain a maximum of 0.8ppm ozone, 2ppm NO2 and 1200ug/m3 PM; however, target concentrations for these pollutants may be adjusted in future studies. Continuous gas and aerosol sampling for CO, O3, NOx, O2, total hydrocarbon (THC) and particle mass concentration will be conducted at both the MRC unit as well as from the inhalation exposure systems. Rats will either be exposed whole-body in wire rack cages, which will be placed in the exposure chamber, or nose-only. NOTE: the mode of exposure will depend on the method that is most optimal for generating the appropriate smog atmosphere. Rats will be exposed within this system to air or a single concentration of the smog atmosphere either for one or two consecutive days. During the exposure periods restrained animals will be restricted from food and water.

During exposures, Rats will be visually inspected for evidence of overt changes in ventilatory or activity patterns. Exposures will take place in the HighBay facility.

HC030031 - TRPA1 antagonist - will be administered intraperitoneally once 15 minutes before smog exposure at a dose of 5mg/kg - NOTE: all injections will be in a volume of 0.5-1.0 ml (ip)

b. Survival Blood Collections (method, volume, frequency):

none

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

none

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

Nose-only inhalation exposure: Prior to inhalation exposure, rats will be acclimatized to conical plastic nose-only inhalation tubes (designed for nose-only inhalation purpose) for 2 days (on day 1, rats will be acclimatized for 15 minutes in the morning from 8am-8:15am and 30 minutes in the afternoon from 4pm to 4:30pm; on day 2 rats will be acclimatized for 1 hour from 8am to 9am). During each acclimation period animals will be restricted from food and water. Animals will be placed in the nose-only restraining tubes on the holding rack during acclimation period. Then rats will be restrained for a period of 4 hours to nose-only tubes during the test of atmosphere exposures for up to 2 days. Four hours exposures in a given day to nose-only tubes have been well tolerated by rats as shown in several of our previous studies.

* Acclimatization of animals will also occur as above if they are exposed whole-body.

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

none

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:

All animals will be identified with a unique number/code (tail marking) and monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily and particularly during the exposure (once every 30 minutes). The personnel responsible for monitoring include: Exemption 6Exemption 6Exemption 6 Exemption 6Exemption 6 Animals will be weighed every 3 days and tracked for sudden weight loss (>10%). If signs of distress or other deleterious effects are observed, all animals from the treatment group

will be isolated in a clean control atmosphere and observed for recovery trends. They may be reused for the study if recovery is demonstrated; otherwise, they will be euthanized.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

none

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

none

c. Testing methods:

none

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

none

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

none

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

none

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

none

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

Intravascular/ventricular (Millar) functional testing: Experiments will be performed 24 hours following the last exposure as described in the regimen above. Rats will be anesthetized with urethane (1.5 g/kg, i.p.) and the area of the neck overlying the left jugular will be clipped of hair and wiped down with alcohol prep. Following assurance of proper anesthetic plane with toe-pinch, a midline incision will be made from 1 cm caudal to the mandibular symphysis to a point just cranial to the sternal manubrium and the left jugular vein will be surgically catheterized with a saline-filled PE50 tubing for administration of drugs. Body temperature will be supported during and after surgery with recirculating warm water blanket.

The right common carotid artery will then be isolated and ligated with 3.0-silk suture just caudal to its bifurcation. A small bulldog clamp will be placed around the artery as close as possible to the sternal manubrium and a second, loose, ligature tied immediately cranial to the bulldog clamp. With iris scissors pointing towards the heart an oblique cut will be made in the ventral surface of the artery 1 cm cranial to the clamp. The Millar probe, which will be connected via a Pressure Control Unit (Model 2000, Millar Instruments) to a receiver (Powerlab 4/30, ADInstruments) and a computer acquiring data at 1000 Hz, will be inserted into the artery and toward the heart with its curvature directed toward the midline to measure pressure. The loose ligature cranial to the clamp will be tightened with a half-hitch knot and the probe advanced after removing the bulldog clamp until resistance is felt at the aortic semilunar valve. To avoid damage if resistance is sensed, the catheter will be slowly withdrawn 2-3 mm, rotated 1/4-1/2 turn clockwise and advanced again toward the ventricle. Proof of entry will be monitored by observing the ventricular pressure wave transmitted via the probe/transducer to a channel recorder. Normal diastolic pressure in a rat is approximately 100 mmHg. This pressure reading goes to zero once the catheter is extended into the ventricle (ventricular pressure is roughly equivalent to carotid systolic pressure). Once ventricular pressure readings are confirmed, a baseline period will be recorded for ten minutes after which freshly-diluted dobutamine (640 ug/ml - dissolved in saline) will be infused intravenously for 2min at a dose of 320 µg/kg/min. Rats will then be monitored for at least 12 minutes thereafter or until heart rate and contractility

are back to resting levels. The administration of dobutamine, a beta adrenergic agonist, causes an increase in cardiac rate and contractility and has been shown by us to elicit adverse cardiac effects after air pollution exposure. Animals will then receive bilateral vagotomy by suture occlusion followed by a stabilization period (3min) and another 2-min infusion of dobutamine at the same dose. The purpose of vagotomy is to sever the parasympathetic influence on the heart and vasculature, thus it would determine whether it plays a role in the adverse effects of exposure.
All animals will be euthanized by a lethal dose of Na pentobarbital: 200 mg/kg; Phenytoin: 25 mg/kg administered i.v. at the end of the procedure.

Aconitine challenge arrhythmia sensitivity test: 24 hours following the last exposure as described in the regimen above, i.v. aconitine challenge will be performed. Rats will be anesthetized with urethane (1.5 g/kg, i.p.) and surgically catheterized into the left jugular vein with a saline-filled PE50 tubing for administration of drugs following assurance of proper anesthetic plane with toe-pinch. The area of the neck overlying the left jugular will be clipped of hair and wiped down with alcohol prep. The experiments will be performed immediately following implantation of the i.v. catheter. Body temperature will be supported during and after surgery with recirculating warm water blanket. 10 micrograms/ml aconitine (in saline) will be infused into the jugular vein at a speed of 0.2ml/min (Li et al. 2007) using a ISMATECH IPC infusion pump while ECG is continuously monitored and timed (ECG will be monitored in these animals using an external telemeter that is attached to the skin). Susceptibility will be measured as the threshold dose of aconitine required to produce ventricular premature beats, ventricular tachycardia, and ventricular fibrillation will be calculated using the following formula:

> Threshold dose (ug/kg) for arrhythmia = 10ug/ml x 0.1ml/min x time required for inducing arrhythmia (min)/body weight (kg) = 1ug/min x time (min)/body weight (kg)

All animals will be euthanized by a lethal dose of Na pentobarbital: 200 mg/kg; Phenytoin: 25 mg/kg administered i.v. at the end of the procedure. A surgical record with time of anesthesia, dosing volumes and unexpected responses will be maintained.

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:
Urethane (1.5 g/kg, ip) for intravascular/ventricular functional test and aconitine arrhythmia test. The preference to use urethane over other anesthetics is because (1) it is a long-lasting anesthetic (suitable for these types of terminal procedures) and it has been used traditionally for these types of investigations (2) it does not inhibit cardiovascular reflexes and neural signalling as much as other anesthetics (Peotta et al. 2001; Hara and Harris 2002) (3) it has minimal effects on blood pressure (Kushwaha et al. 2011). Urethane is not available as a pharmaceutical-grade compounds. Although pharmaceutical-grade alternative anesthetics are available, urethane still has an important role as an anesthetic agent in biomedical research due to unique physiologic effects (for example, urethane has minimal respiratory effects).

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):
none

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):
n/a

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?
○ Yes ● No

f. Identify any surgical procedures performed at other institutions or by vendors:
none

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.
Smog may cause dyspneic breathing due to airways irritation; these effects typically stop upon termination of the exposure. Animals also may become lethargic.

b. State the criteria for determining temporary or permanent removal of animals from the study.

Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

One or more of the following: weight loss (> 10%), labored breathing (temporary or persistent), abnormal gait or loss of appetite. The attending veterinarian will be consulted when appropriate for suitable treatment and options for inclusion in the study.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

Intravenous infusion is the only method available that will allow metered dosing of dobutamine or aconitine with direct access to the heart. Intravascular/ventricular catheterization with a pressure (Millar) probe is the only way to measure ventricular mechanical function in the whole animal. (based on a PubMEd search dated 3/28/16 containing the following key words: aconitine, dobutamine, cardiac, arrhythmia, infusion, rats, left ventricular pressure).

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study:	1792
b. Animals to be transferred from another LAPR:	0
LAPR Number that is the source of this transfer:	
c. Animals to be transferred from another source:	0
d. Offspring produced onsite (used for data collection and/or weaned):	0
e. TOTAL NUMBER of animals for duration of the LAPR	1792

2. Species (limited to one per LAPR): Rat(s)

3. Strain: WKY rat(s) and SH rats

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

none

4. Sources of animals:

Charles River Laboratories

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6
Exemption 6
- Terminal surgery for intravascular/ventricular function and aconitine challenge tests, Euthanasia (only animals that did not leave A-bldg)

Exemption 6 (Animal Care Facility)
- Housing

Exemption 6
Exemption 6
Exemption 6
- Terminal surgery for intravascular/ventricular function and aconitine challenge tests, euthanasia, necropsies of Highbay animals

Exemption 6
- approved satellite housing and exposure (smog) area; inhibitor injections

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

Yes. Rats will be transferred to the approved satellite housing/exposure facility Exemption 6 for smog exposures. Individuals working in Exemption 6 during studies will not go back into Building A animal facility that day without a shower and complete change of clothes.

Room Numbers: Exemption 6

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

Standard filter-top polycarbonate cages with pine shavings bedding and a water bottle should be used for transfer between EPA buildings

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

none

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

none

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

The animals will be housed two per cage with any Enviro Dri bedding enrichment.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

Air Pollutants
1. Smog - no LD50 available (maximum = 0.8ppm ozone; 2ppm NO2; 1200ug/m3 particulate matter)

Analgesics/Anesthetics:
1. Sodium pentobarbital/phenytoin: Maximum dose of Na pentobarbital to be admistered = 200 mg/kg; Maximum dose of Phenytoin to be administered = 25 mg/kg). Pentobarbital LD50 rat, oral = 118 mg/kg. rat). Phenytoin LD50 rat, oral = 1530 mg/kg

2. Urethane: Maximum dose to be administered is 1.5 mg/kg. LD50 rat, oral = 1809 mg/kg
* The will be a fresh working solution (daily)

Other drugs:
1. Aconitine (C34H47NO11): LD50 in rats = 5.97 mg/kg (oral); 80 ug/kg (intravenous). Maximum dose =

60 ug/kg (intravenous). HSRP: Title: "ARRHYTHMIA SUSCEPTIBILITY FOLLOWING EXPOSURE TO AIR TOXICS IN WISTAR-KYOTO, SPRAGUE DAWLEY AND SPONTANEOUSLY HYPERTENSIVE RATS"

2. Dobutamine: LD50 in rats = 500 mg/kg (Oral); 92 mg/kg (intravenous). Maximum dose = 1.28 mg/kg (intravenous).

3. Saline (Pharmaceutical grade): a non-hazardous agent used as a vehicle for drugs used for cardiovascular challenge; maximum dose = 500 microliters.

4. HC030031: TRPA1 antagonist. No LD50 available. Maximun dose = 10mg/kg (intraperitoneal).

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.

Urethane and aconitine are not available as a pharmaceutical grade - all other drugs are pharmaceutical grade.

Components of smog are environmental agents and are thus not available in pharmaceutical grade.

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

none

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

n/a

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	study coordination, surgical procedures; physiological monitoring during experimentation, arrhythmia testing	14 years of experience in use of laboratory animals; 12 years of experience in surgical procedures and physiological monitoring; completed NHEERL training
Exemption 6	Associate Principal	study coordination, surgical procedures;	14 years of experience in use of laboratory animals; 12 years of experience in surgical

	Investigator	physiological monitoring during experimentation, arrhythmia testing	procedures and physiological monitoring; completed NHEERL training
Exemption 6	Student	surgical procedures; physiological monitoring during experimentation, arrhythmia testing	2 years of experience in use of laboratory animals, surgical procedures and physiological monitoring; completed NHEERL training
Exemption 6 Exemption 6 Exemption 6	Student	surgical procedures; physiological monitoring during experimentation, arrhythmia testing	5 years of experience in use of laboratory animals, surgical procedures and physiological monitoring; completed NHEERL training
Exemption 6	Technical Staff	animal handling/care; surgical procedures; physiological monitoring via radiotelemetry	>20 years of experience in use of laboratory animals and pulmonary function monitoring; 6 years, surgical procedures; completed NHEERL training
Exemption 6	Post-Doc	animal handling/care; surgical procedures; physiological monitoring via radiotelemetry	8 years of experience in use of laboratory animals, surgical procedures and physiological monitoring; completed NHEERL training
Exemption 6	Student	animal handling/care; physiological monitoring via radiotelemetry	<2 years of experience in use of laboratory animals and physiological monitoring; completed NHEERL training. She will be mentored/monitored in all procedures by the principal investigator.
Exemption 6	Technical Staff	animal handling; inhalation procedures	>20 years of experience in use of laboratory animals and inhalation exposures; has completed NHEERL training
Exemption 6	Technical Staff	animal handling; inhalation procedures	>20 years of experience in use of laboratory animals and inhalation exposures; has completed NHEERL training
Exemption 6	Technical Staff	animal handling; inhalation procedures	>20 years of experience in use of laboratory animals and inhalation exposures; has completed NHEERL training
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year 0

2. Breeding protocols and recordkeeping 0

3. Methods for monitoring genetic stability 0

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR 0

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

All animals will be euthanized following completion of experimental procedures (up to 24 hours following last exposure)

2. Describe the euthanasia techniques:

Method(s): Euthanasia plus exsanguination, Anesthesia plus vital organ transsection
Agent(s): Sodium pentobarbital: 200 mg/kg; Phenytoin: 25 mg/kg)
Dose (mg/kg): overdose of pentobarbital (150-250 mg/kg) followed by transection of abdominal aorta and vital organs
Volume: Approximately 0.25 ml/rat for a 250 g rat (200 mg/ml solution)
Route: Intraperitoneal

Source(s) of information used to select the above agents/methods:

2013 AVMA Guidelines on Euthanasia.

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

n/a

4. Describe how death is to be confirmed.

Vital organ section

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

Euthanized as above
Euthanized by Animal Care Contractor
Transferred to another study

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.

7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	03/30/2016

Submitted: 03/30/2016

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	***Approval Date***	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	03/30/2016 by Exemption 6 Exemption 6/RTP/USEPA/US	Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6/RTP/USEPA/US	EPHD ***Branch*** CIB	MD ***Submitted to Branch Chief for Approval*** 03/30/2016 11:55 AM

ATTACHMENTS

PI resp new lapr.pdf 19-04-001 SHEM resp.pdf

Actions

First Update notification sent: 03/02/2017
Second Update notification sent: 04/03/2017
First 2nd Annual notification sent: 03/02/2018
Second 2nd Annual notification sent: 04/26/2018
1st Expiration notification sent:
2nd Expiration notification sent:

History Log: